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Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building

Abstract We investigated the associations of fungal and endotoxin levels in office dust with respiratory health in 888 (67% participation) occupants of a water-damaged building. We analyzed floor and chair dusts from 338 workstations for culturable fungi and endotoxin. Based on averages, we ranked each floor of the building as low, medium, or high for occupants' exposure to each of these agents. Multivariate logistic regression models for building-related symptoms included this ranking of fungi and endotoxin, age, gender, race, smoking status, and duration of occupancy. Using floor dust measures, we found significantly increased odds for lower respiratory symptoms [wheeze, chest tightness, attacks of shortness of breath, and attacks of cough: odds ratios (OR) = 1.7 (95% confidence interval (CI): 1.02–2.77) to 2.4 (95% CI: 1.29–4.59)], throat irritation [OR = 1.7, (95% CI: 1.06–2.82)], and rash/itchy skin [OR = 3.0, (95% CI: 1.47–6.19)] in the highest fungal exposure group compared to the lowest, with generally linear exposure–response relationships. Nonlinear relationships were observed for many of these symptoms and endotoxin in floor dust. Interaction models showed that endotoxin modified effects of fungi on respiratory symptoms. Our findings of exposure interactions and exposure–response relationships of fungal and endotoxin with increased risk of building-related symptoms contribute to an understanding of the role of microbial agents in building-related asthma and respiratory and systemic symptoms.

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Key words: Bioaerosol; Endotoxin; Exposure–Response Relations; Fungi; Office Building; Respiratory Symptoms; Water Damage.

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Practical Implications
Our demonstration of exposure–response relationships between measurements of fungi and/or endotoxin in floor dusts and building-related symptoms implies that microbial agents in floor dust may be a good surrogate measure for dampness-related bioaerosol exposure, considering that measurements of microbial agents in air often fail to demonstrate the associations between exposure and health. In addition, our finding that endotoxin exposure may change the effect of fungal exposure (and vice versa) on respiratory heath suggests that exposure to both fungi and endotoxin should be assessed in epidemiological investigations examining the effect of fungal or endotoxin exposure on respiratory health in indoor environments.

Introduction

Exposure to dampness and mold in indoor environments has been recognized as a public health hazard, and associations with lower and upper respiratory symptoms and respiratory illnesses in adults have been reported (Jaakkola et al., 2002; Park et al., 2004). Recently, the committee on damp indoor spaces and health for the Institute of Medicine (IOM) concluded that there is sufficient evidence of associations of building dampness and presence of mold in damp indoor environments with nasal and throat symptoms, wheeze, cough, and asthma symptoms in sensitized people; that there is suggestive evidence of associations with shortness of breath and development of asthma; and that there is inadequate or insufficient evidence of associations with skin symptoms and fatigue (Institute of Medicine of the National Academies of Science (IOM), 2004).

For most studies showing these associations, the methods of exposure assessment to mold were signs of dampness and presence of visible mold (IOM, 2004; Kolstad et al., 2002). Findings for associations between objective measurements of fungi in indoor environments and respiratory diseases or symptoms in adults have been inconsistent (Chao et al., 2003; Li et al., 1997; Nelson et al., 1995; Wan and Li, 1999). The associations of dampness and presence of visible mold

with various respiratory symptoms do not necessarily mean that mold is the etiologic agent of these illnesses. However, biologically derived airborne contaminants, including mold, related to building dampness have been suspected as potential causative agents for asthma and symptoms.

Associations have been reported for house dust endotoxin levels with repeated wheeze in infants (Keman et al., 1998; Park et al., 2001) and increased severity of asthma in adults sensitized to dust mite (Michel et al., 1991, 1996). Rose and coworkers reported hypersensitivity pneumonitis among lifeguards of an indoor swimming pool after exposure to high levels of airborne endotoxin, and suggested that endotoxin may be an etiologic agent, a marker of unmeasured agents, or an immunologic adjuvant of the disease (Rose et al., 1998). However, only limited data exist on the association of endotoxin exposure with respiratory symptoms among adults in non-industrial work environments (Gyntelberg et al., 1994; Teeuw et al., 1994; Wan and Li, 1999), although environmental endotoxin is a pervasive, potent pro-inflammatory agent (Keman et al., 1998; Park et al., 2001). We are not aware of reports of an interaction effect between fungi and endotoxin exposures in epidemiological studies of the indoor environment.

The National Institute for Occupational Safety and Health (NIOSH) conducted an epidemiological study in 2001–2002 in a large office building with a history of water-damage. In the study, we examined whether a group of occupants of floors with higher levels of fungi and endotoxin in floor dust samples would show elevated prevalence of building-related respiratory and other symptoms in an exposure-dependent manner. We also examined if endotoxin exposure modifies the effects of fungal exposure on the reported symptoms.

Background

The study building is a large 20-story office building constructed in 1985 and located in a metropolitan area in the north-east United States. The building is primarily cubicle office space on floors 6–20, with open-air parking garages on the first four floors and a lobby/cafeteria/mezzanine area with some office space on the fifth floor. A history of water damage and remediation actions in the building has been described in detail elsewhere (Cox-Ganser et al., 2005). Within a few months of building occupancy in 1994, employees perceived new-onset respiratory and dermatological conditions to be building-related, and they complained of an increase in symptom severity and frequency beginning in the fall of 2000. Sentinel cases of post-occupancy onset asthma ($n = 67$), hypersensitivity pneumonitis ($n = 8$), and sarcoidosis ($n = 6$) had been diagnosed and relocated to another facility.

The NIOSH conducted a building-wide questionnaire survey in September 2001. Of 1327 employees working in the building, 888 (67%) participated in a self-administered questionnaire. Prevalence of wheezing, lifetime asthma, and current asthma were 2.2 to 2.5 times those in the U.S. adult population as found in the Third National Health and Nutrition Examination Survey (NCHS, 1996). Adult-onset asthma was 3.3 times higher than that in the U.S. adult population; reported wheeze, nasal, or eye symptoms improving away from work were 3.4 times higher in the study building than in the U.S. adult population. The incidence density of adult-onset asthma after building occupancy was 7.5 times higher than that before occupancy. More than 50% of the asymptomatic participants in the September 2001 survey reported respiratory symptoms in a survey 9 months later, consistent with continued incidence of building-related illness (Cox-Ganser et al., 2005).

Materials and methods

Epidemiological investigation

For the present study, we analyzed the September 2001 questionnaire data on respiratory and other symptoms in the past 12 months to assess exposure–response relationships. The analyzed questionnaire data included demographics, upper and lower respiratory symptoms, systemic symptoms, and skin conditions occurring in the last 12 months, building-relatedness of those symptoms, and post-occupancy onset of asthma with use of asthma medications in the past 12 months. We defined building-related symptoms as those that improved when away from the building (i.e. over the weekend or during a holiday or vacation).

Environmental sampling

In April 2002, we collected dust samples from carpeted floors and chairs of 356 workstations on all 15 occupied floors of the building. We sampled 202 workstations of all employees reporting a defined set of lower respiratory and/or systemic symptoms in September 2001, and 154 workstations of all employees with no such symptoms, based on an original case–comparison study design (Cox-Ganser et al., 2005). We used the environmental measurements taken at these 356 sampling locations to categorize exposure for all 888 respondents of the September questionnaire survey.

Floor and chair dust were collected onto polyethylene filter socks (Midwest Filtration Company, Fairfield, OH, USA) with a crevice tool and a L'il Hummer™ backpack vacuum (100 CFM, 1.5 horse power, Pro-Team Inc., Boise, ID, USA). Each crevice tool for an individual sampling location was cleaned with isopropyl

alcohol before sampling. For each sampling location, one chair was vacuumed for 3 min and a 2 square meter (m^2) carpeted-floor area was vacuumed for 5 min using different crevice tools. The samples were sealed in plastic bags and transported to the laboratory where collected dusts in the filter socks were emptied into 50 ml pyrogen-free conical tubes and homogenized by rotation on a 360-degree rotary arm shaker at 65 r.p.m. for 2 h. Hair, fluff, and other larger objects were removed from the sample before homogenization. The dust samples were then weighed, partitioned, and sent for analyses of culturable fungi, endotoxin, cat allergen, and dog allergen. The samples for fungi were cultured with malt extract, dichloran glycerol 18, and cellulose agars at room temperature for 7–10 days. Total culturable fungi were reported as colony forming units (cfu) per milligram dust and also per square meter (for floor samples) or per chair (for chair samples) by multiplying the resulting cfu per milligram value by the total amount of dust collected in each floor (or chair) sample and then dividing by 2 m^2 for floor samples. Endotoxin samples were analyzed with the Limulus amoebocyte lysate assay using kinetic QCL methods (Chun et al., 2002), and the results were reported as endotoxin units (EU) per milligram dust, and also as per square meter or per chair. Dog and cat allergens were analyzed with an enzyme-linked immunosorbent assay, and reported as micrograms per milligram dust, and also as per square meter or per chair.

Floor categorization for exposure variables in statistical modeling

Because we did not have individual exposure measurements for all 888 occupants, we categorized all participants into dichotomous or tertile exposure groups based on the floor they occupied. We estimated floor-specific geometric mean (GM) exposures for culturable fungi, endotoxin, and cat and dog allergens in dust samples. Based on rank order of these floor-specific means, the 15 floors were categorized into three exposure groups: low (first tertile), medium (second tertile) or high (third tertile) exposure floors for each of the four agents in each unit of measurements (per milligram or per square meter for floor dust samples; per milligram or per chair for chair dust samples). For the models using dichotomous exposure variables, floors in the upper two tertiles were classified together as the high exposure group. We chose this categorization because, in the no-interaction multivariate models on health outcomes, the major increase in risk for endotoxin exposure was generally between the first tertile and the upper two tertiles.

Data analysis

We computed GM and geometric standard deviations (GSD) for culturable fungi and endotoxin from log-transformed data because of right-skewed distributions. The log-transformed measurements were also used for statistical analyses. Because of the large GSD, we assigned a value of limit of detection (LOD)/2 to four samples below the LOD for culturable fungi in dust (Hornung and Reed, 1990). The LOD was between 350 and 400 cfu/g, depending on amount of dust analyzed.

We used multivariate logistic regressions (SAS version 8.02, SAS Institute, Cary, NC, USA) to estimate odds ratios (ORs) of building-related respiratory and other symptoms for fungal (or endotoxin) exposure after adjusting for endotoxin (or fungal) exposure and demographic factors: age, gender, race/ethnicity, smoking status, and duration of building occupancy. We determined the final multivariate logistic regression models by examining changes in ORs and precision [95% confidence interval (CI)] in reduced models, and by the log likelihood ratio tests compared to full models including demographic factors and environmental exposure variables (fungi, endotoxin, and dog and cat allergens in floor dusts, and fungi and endotoxin in chair dusts; Kleinbaum and Klein, 2002). The final models included fungi and endotoxin in floor dust and demographic factors. We further assessed the final models with Hosmer and Lemeshow Goodness-of-Fit test statistics (Hosmer and Lemeshow, 2000), and test results showed that the null hypothesis that the fitted final models are appropriate was not rejected ($P > 0.05$). With the final models, we tested interaction effects between fungi and endotoxin exposure using the product of the two variables for all symptoms. To estimate ORs of those symptoms for each combined level of exposure to fungi and endotoxin, we created a categorical interaction variable by combining dichotomous exposure variables of fungi and endotoxin: high fungi/high endotoxin floors; high fungi/low endotoxin floors; low fungi/high endotoxin floors; and low fungi/low endotoxin floors (reference exposure group). We computed Pearson correlation coefficients (SAS version 8.02) to examine correlations between the fungal and, separately, endotoxin levels in floor and chair dust.

Results

Among the 888 respondents, 74% were white and 59% were female. Only 14% were current smokers and 62% of the respondents never smoked. Their average age was 46 years old (s.d. 8.7) and, on average, they had been working in the building for 6 years (Cox-Ganser et al., 2005). One-third or more reported wheeze, chest tightness, or shortness of breath in the past 12 months. Most respondents (79%) reported nasal symptoms, and more than half reported other upper respiratory and mucous membrane symptoms (Figure 1). Generally, more than half of these symptomatic respondents

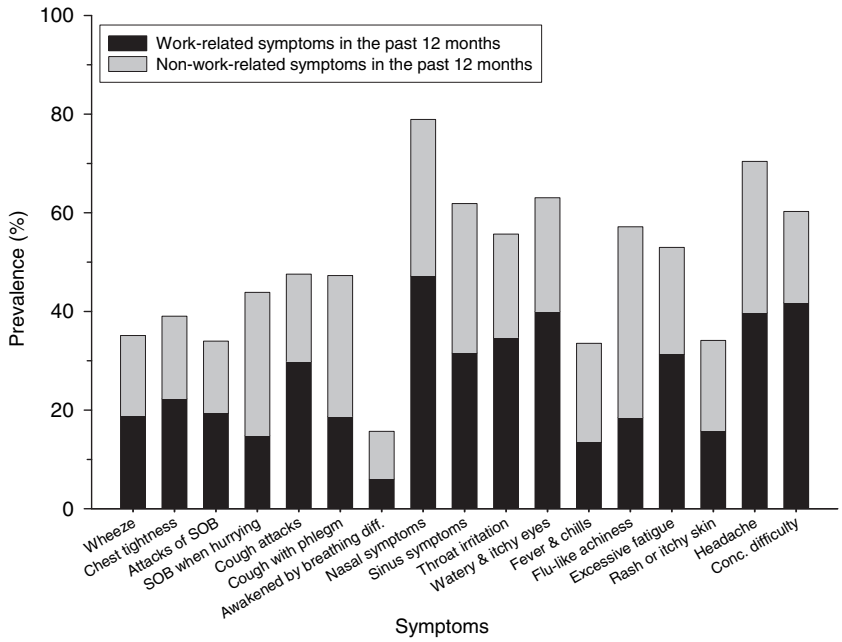

Fig. 1 Prevalence of work-related and non-work related symptoms in the past 12 months. Work-related symptoms were defined as 'symptoms which were better away from the building.' SOB: attacks of shortness of breath; Conc. difficulty: concentration difficulty

reported symptom improvement when away from work. Rash or itchy skin was reported by 34% of the respondents, and 46% of skin symptoms were building-related. The prevalence of physician-diagnosed asthma with onset after occupancy was 7.6%, and three-quarters of those who reported asthma with post-occupancy onset still had asthma at the time of the study.

After excluding missing, mislabeled, and insufficient dust samples, we had 338 samples available for analysis. Table 1 presents the number of dust samples by floor and categorization of floors based on floor-specific GMs of culturable fungi and endotoxin. The arithmetic means and ranges of floor-specific GM levels of total culturable fungi and endotoxin in floor dust within each tertile category are also presented in Table 2 for both units. The overall level of fungi per milligram of floor dust was significantly ($P = 0.004$) lower than that of chair dust (Table 3). The concentration of endotoxin per mg floor dust was significantly ($P < 0.001$) higher than that in chair dust (Table 3). The levels of total culturable fungi in floor dust were significantly ($P < 0.001$) but weakly correlated with those in chair dust ($r = 0.20$ for cfu/mg, 0.22 for cfu/m^2 or cfu/chair; $n = 314$); however, the levels of endotoxin in floor dust were not correlated with those

Table 1 Number of samples analyzed and categorization of floors based on the geometric mean levels of culturable fungi (cfu/m^2) and endotoxin (EU/m^2) in floor dust

Floor of the building	Number of dust samples collected	Culturable fungi		Endotoxin	
		Tertile category	Geometric mean	Tertile category	Geometric mean
5[a]	4	Low	610	High	4540
6	30	Medium	2060	Medium	2660
7	30	Medium	1830	Low	1210
8	21	Low	1170	Low	1000
9	26	Medium	1770	High	7610
10	28	Low	910	Medium	1800
11	16	Medium	1380	Low	620
12	19	Low	590	Low	500
14	30	High	3120	Medium	1430
15	27	High	7760	High	10,370
16	8	High	5830	Medium	2720
17	58	High	2180	High	6890
18	20	High	3210	High	4070
19	20	Medium	2040	Medium	3300
20[a]	1	Low	760	Low	200
Total	338	–	–	–	–

[a]Fifth and twentieth floors had only 27 and seven occupants, respectively. All other floors had more than 50 occupants.

Table 2 Arithmetic means and ranges of floor-specific geometric mean levels of culturable fungi and endotoxin in floor dust within each exposure category

	Tertiles of exposure		
Unit of measurements	Low ($n = 5$)	Medium ($n = 5$)	High ($n = 5$)
Average levels (ranges) of culturable fungi			
cfu/mg floor dust	4.9 (3.9–5.8)	7.9 (6.2–8.9)	12.5 (10.0–21.6)
cfu/m^2 floor area	800 (600–1200)	1800 (1400–2100)	4400 (2200–7800)
Average levels (ranges) of endotoxin			
EU/mg floor dust	3.5 (2.3–4.6)	8.1 (5.7–10.5)	33.7 (12.7–65.6)
EU/m^2 floor area	700 (200–1200)	2400 (1800–3300)	6700 (4100–10,400)

cfu, colony forming unit; EU, endotoxin unit.

in chair dust (Figure 2). The levels of total culturable fungi were significantly (P-values < 0.001) but weakly correlated with those of endotoxin, both per mg ($r = 0.27$ in floor dust, 0.28 in chair dust) and per area or chair ($r = 0.38$ in floor dust, 0.37 in chair dust) [data not shown].

In our multivariate logistic regression models including both fungi and endotoxin in floor dust and those in chair dust, chair fungi and endotoxin did not significantly contribute to the models. We generally found linear exposure–response relationships between fungal exposure based on the cfu/m^2 in floor dust and building-related upper and lower respiratory symptoms in final multivariate models adjusted for endotoxin exposure (EU/m^2), age, gender, race, smoking status, and duration of occupancy in the building (Figure 3). The ORs of building-related lower respiratory symptoms (wheeze, chest tightness, attacks of shortness of breath, attacks of cough, and cough with phlegm) significantly increased in an exposure-dependent manner for second and third tertile categories of fungal exposure based on cfu/m^2. The ORs ranged from 1.1 to 1.7 for the second tertile category of fungal exposure, and 1.7–2.4 for the third tertile exposure category (Figure 3). We also observed similar linear trends for building-related nasal symptoms and throat irritation. Strong linear trends in ORs for rash and itchy skin were also seen. However, a similar linear trend was not observed for the symptom 'awakened at night by breathing difficulty,' although odds for both second and third tertiles were significantly increased. We observed only weak linear trends of ORs for hypersensitivity pneumonitis-like symptoms (shortness of breath when hurrying on level ground or walking up a slight hill, and fever and chills; Figure 3). However, we did not find significant associations of flu-like achiness, excessive fatigue, or loss of concentration with fungal exposure. We also observed significantly increased risks of lower respiratory symptoms for second and/or third tertiles of fungal exposure based on cfu/mg floor dust, but we found no apparent exposure–response relationship as those found based on cfu/m^2. No significantly increased risks of upper respiratory, systemic, and skin symptoms were observed in the same cfu/mg floor dust-based models [data not shown].

We found significantly increased odds for all lower (OR = 1.6–2.2) and upper (OR = 1.5–2.0) respiratory symptoms, fever and chills (OR = 2.2), and headache (OR = 2.0) in the second tertile of endotoxin exposure based on EU/m^2 floor in multivariate models adjusted for fungal exposure (cfu/m^2) and the demographic factors (Figure 4). The odds in the third tertile exposure for most of those symptoms were similar or somewhat lower but remained greater than those in the first tertile exposure (reference) group; they were still significant for building-related wheeze, chest tightness, shortness of breath when hurrying on level ground or walking up a slight hill, awakening at night by breathing difficulty, fever and chills, headache, and nasal symptoms. We did not find significant associations of flu-like achiness, excessive fatigue, or loss of concentration with endotoxin exposure. We observed increased odds (1.8 and 1.6 for second and third tertiles, respectively) and the same nonlinear trends for post-occupancy onset of asthma with asthma medications in the past 12 months, but the ORs were not significant. We did not find significant associations of respiratory symptoms with exposure tertiles based on EU/mg dust.

We found interaction effects between endotoxin and fungal exposure on lower respiratory symptoms (attacks of shortness of breath, $P = 0.01$; shortness of breath when hurrying, $P = 0.04$). The interaction

Table 3 Distribution of levels of culturable fungi and endotoxin in floor and chair dust samples

	Fungi				Endotoxin			
	Floor dust		Chair dust		Floor dust		Chair dust	
Parameters	cfu/mg	cfu/m^2	cfu/mg	cfu/chair	EU/mg	EU/m^2	EU/mg	EU/chair
Total number of samples	328		329		338		327	
<LOD	3		1		0		0	
Minimum	0.2	32	0.2	112	0.03	15.2	0.02	6.3
Maximum	1200	8.7×10^5	5900	1.8×10^6	450	2.3×10^5	24	5.2×10^4
Median	6.7	1.7×10^3	7.9	3.8×10^3	9.9	2.5×10^3	0.3	3.0
GM	7.7	2.0×10^3	11.3	5.1×10^3	10.9	2.7×10^3	2.1	900
GSD	4.7	5.5	5.5	5.9	4.1	4.8	2.6	3.0

LOD, limit of detection; GM, geometric mean; GSD, geometric standard deviation; cfu, colony forming unit; EU, endotoxin unit.

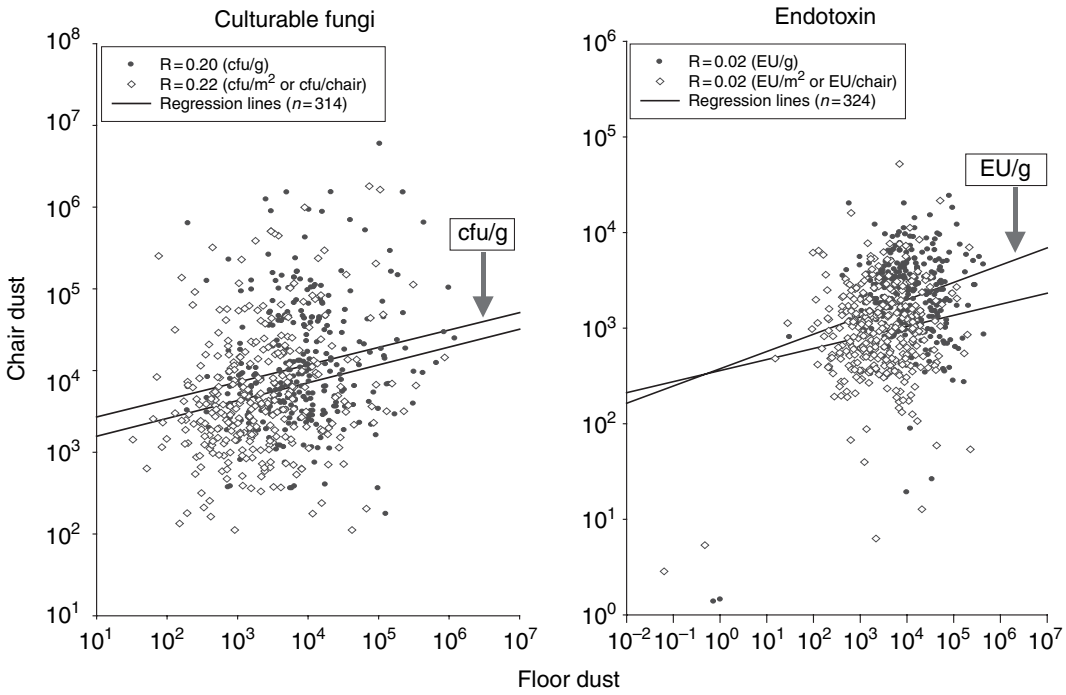

Fig. 2 Correlations between floor dust and chair dust for fungi (a) and endotoxin (b). Closed circles denote cfu (or EU) per gram of culturable fungi (or endotoxin) in floor or chair dust, and open diamonds denote cfu (or EU) per square meter or per chair of culturable fungi (endotoxin) in floor or chair dust

models with ordinal exposure variables created by the combination of dichotomous fungi and endotoxin variables based on cfu/m^2 and EU/m^2 showed that the apparent increased risks of lower respiratory symptoms due to high fungal (or endotoxin) exposure compared with low fungal (or endotoxin) exposure among the employees with high endotoxin (or fungi) exposure were substantially different from those among the employees with low endotoxin (or fungi) exposure (Table 4). There seemed to be no effects of fungal exposure on respiratory symptoms when the endotoxin exposure levels were low. However, among employees with low fungal exposure there appeared to be somewhat increased effects of endotoxin exposure on wheeze, chest tightness, cough with phlegm, nasal symptoms, and throat irritation. For employees with both high fungal exposure and high endotoxin exposure compared to those with low exposure in both, the apparent risks were significantly increased by 2.3–3.8 times for lower respiratory symptoms, 2.6 times for nasal symptoms, and 2.2 times for throat irritation.

Discussion

Association of fungal and endotoxin exposure with respiratory and skin illnesses

Our study findings indicate that both fungal and endotoxin exposures in this damp office-building environment may be important contributors to building-related respiratory symptoms characteristic of asthma, hypersensitivity pneumonitis, and rhinitis. Alternatively, culturable fungi and endotoxin may be surrogates for causal agents related to dampness that remains unmeasured. Our findings of exposure–response relationships for fungi and endotoxin contribute to a causal interpretation of these exposures on wheezing, chest tightness, attacks of shortness of breath, cough attacks, and throat irritation, and thus address research gaps concerning causal relationships that were identified in the recent IOM review (IOM, 2004). This building population had a striking increase in asthma incidence density after building occupancy and continuing incidence of respiratory symptoms among the asymptomatic during the months preceding environmental characterization (Cox-Ganser et al., 2005). These findings are consistent with a temporal association between environmental exposure in the building and the observed excesses of respiratory symptoms, asthma, and possibly also hypersensitivity pneumonitis in these office workers.

Our study addresses another gap identified by the IOM report, which found insufficient evidence for any association between mold or dampness and skin symptoms (IOM, 2004). We found a very strong quantitative association between fungal measurements in dust and rash and/or itchy skin, meeting some of the criteria for a causal association. Biologic plausibility, temporal association, and consistency among studies of

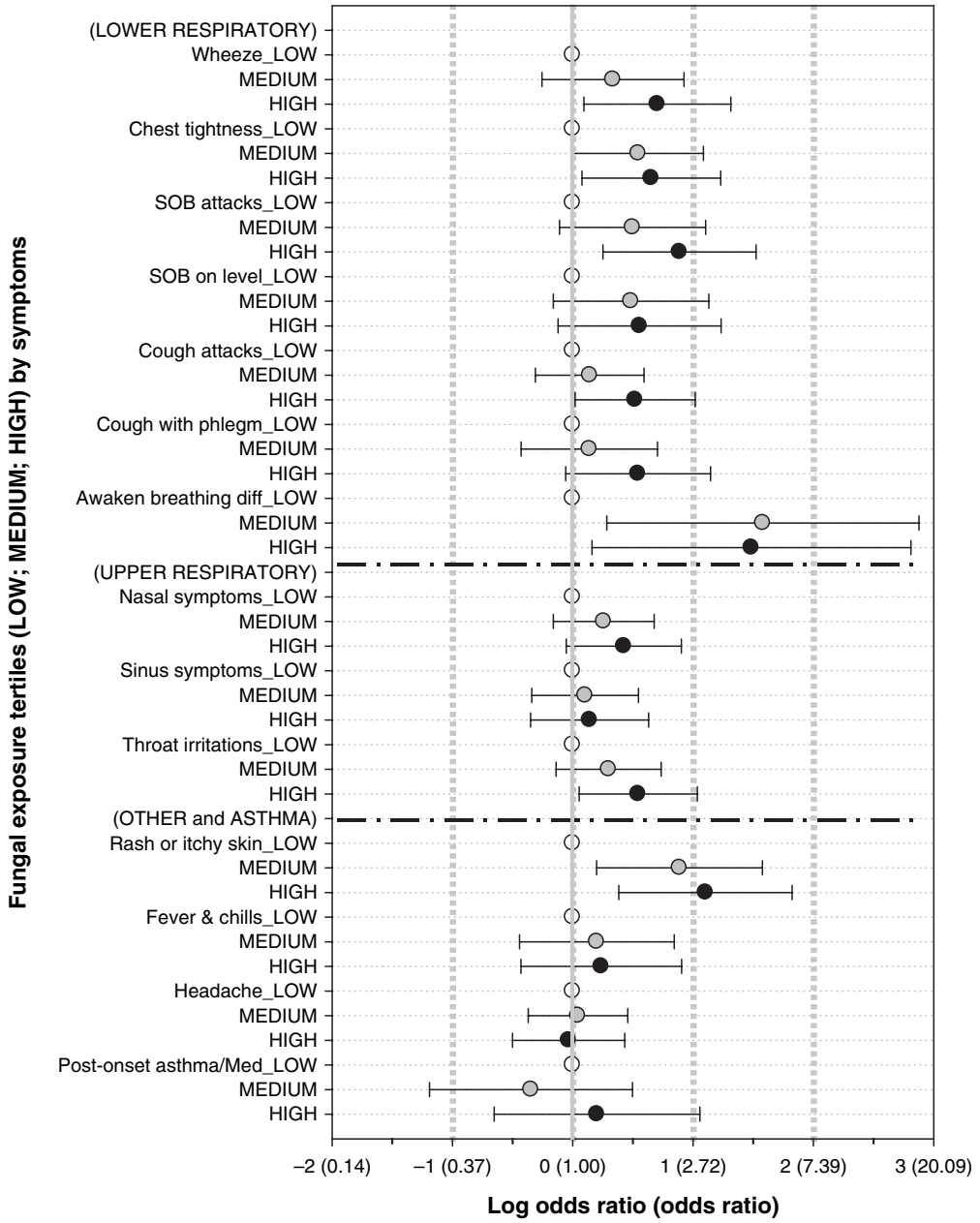

Fig. 3 Odds ratios for symptoms in the past 12 months by tertile exposure to total fungi (based on cfu/m^2 floor). Symptoms presented are grouped into lower respiratory symptoms, upper respiratory symptoms, and other symptoms and asthma. Open circle denotes odds ratio (OR = 1) for reference group (the lowest tertile) for each symptom. ORs are adjusted for endotoxin exposure, age, gender, race/ethnicity, smoking status, and duration of building occupancy. SOB: shortness of breath. Post-onset asthma/Med: post-occupancy onset of asthma with use of asthma medications

skin symptoms remain to be addressed by the indoor air research community.

Assessment of exposure to fungi and endotoxin

Our study addressed many of the pitfalls in exposure assessment which may have been responsible for the several negative studies on the associations of quantitative measurements of microbial agents in damp building environments with respiratory health. Airborne fungi and endotoxin concentrations have large temporal and spatial variability in indoor and outdoor environments (Douwes and Pearce, 2003; Park et al., 2000). This large inherent variability of bioaerosols, paired with commonly used short-term air sampling methods, a limited number of samples, and poor reproducibility of analytical methods, substantially contributes to potential misclassification of exposure in epidemiological studies (Skov et al., 1990; Wan and Li, 1999). We avoided the limitations of airborne

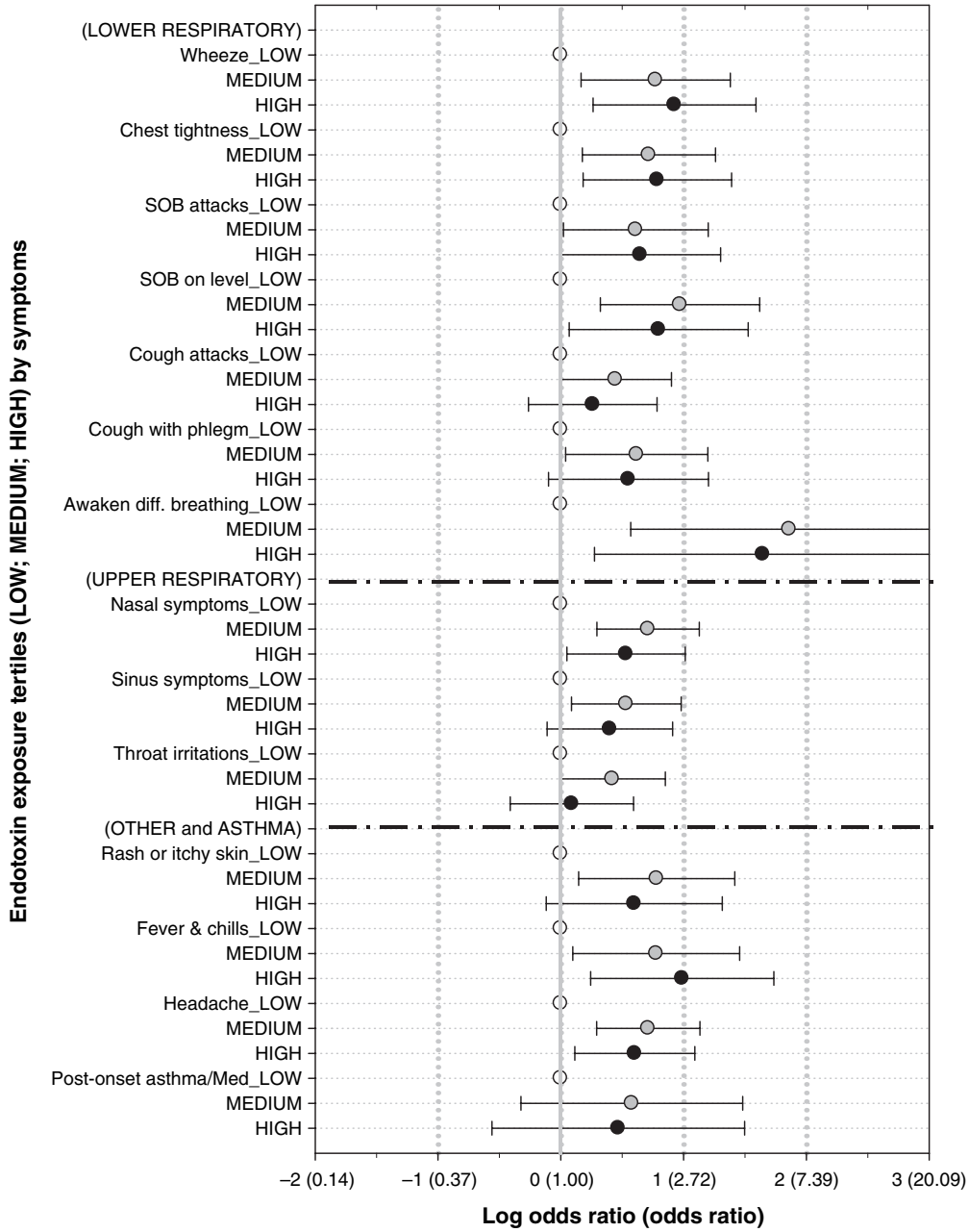

Fig. 4 Odds ratios for symptoms in the past 12 months by tertile exposure to endotoxin (based on EU/m^2 floor). Symptoms presented are grouped into lower respiratory symptoms, upper respiratory symptoms, and other symptoms and asthma. Open circle denotes odds ratio (OR = 1) for reference group (the lowest tertile) for each symptom. ORs are adjusted for fungal exposure, age, gender, race/ethnicity, smoking status, and duration of building occupancy. SOB: shortness of breath; Post-onset asthma/Med: post-occupancy onset of asthma with use of asthma medications

bioaerosol measurements by basing exposure on settled dust samples. Fungi and endotoxin in settled dust may be more representative of longer-term exposure because fungal spores, bacteria or their fragments settle from air over a long time period (Chew et al., 2003). Potential misclassification in our exposure assessment methods using vacuumed dust was also minimized by collecting a large number of dust samples (338 samples) to categorize 888 participants' exposures. This contrasts with approaches used by other investigators who sometimes have collected only one air or settled dust sample per building for exposure assessment (Skov et al., 1990; Wan and Li, 1999).

The levels of culturable fungi and endotoxin in floor dust in our study were lower than other published results (Chao et al., 2001; Hines et al., 2000). However, absolute comparison of the levels of culturable fungi and endotoxin in dust in our study may not be appropriate because sampling/analytical methods are not comparable among studies. Our observed

Table 4 Interaction effect of exposure to culturable fungi and endotoxin in floor dust on work-related lower and upper respiratory symptoms

	No interaction models[a]		Interaction models[b]		
			Exposure to[c]		
Symptoms	Exposure[c]	OR (95% CI)	Fungi	Endotoxin	OR (95% CI)
Wheeze			High	Low	1.2 (0.43–3.35)
	Fungi	1.8 (1.02–3.00)	Low	High	1.9 (0.68–5.33)
	Endotoxin	2.8 (1.62–4.81)	High	High	3.8 (1.59–9.16)
Chest tightness*			High	Low	1.1 (0.46–2.69)
	Fungi	1.8 (1.12–3.04)	Low	High	1.3 (0.52–3.25)
	Endotoxin	2.2 (1.37–3.63)	High	High	3.0 (1.42–6.32)
Attacks of shortness of breath**			High	Low	0.7 (0.27–1.77)
	Fungi	2.0 (1.14–3.51)	Low	High	0.7 (0.25–1.93)
	Endotoxin	2.3 (1.35–3.85)	High	High	2.4 (1.13–5.07)
Shortness of breath when hurrying**			High	Low	0.6 (0.21–1.75)
	Fungi	1.6 (0.89–2.93)	Low	High	0.9 (0.32–2.73)
	Endotoxin	2.5 (1.40–4.57)	High	High	2.3 (0.99–5.24)
Cough with phlegm			High	Low	1.2 (0.46–3.16)
	Fungi	1.4 (0.82–2.30)	Low	High	1.9 (0.73–5.00)
	Endotoxin	2.2 (1.30–3.65)	High	High	2.7 (1.20–6.27)
Stuffy, itchy, runny nose, sneezing			High	Low	1.3 (0.66–2.38)
	Fungi	1.3 (0.90–1.96)	Low	High	1.9 (0.96–3.75)
	Endotoxin	2.0 (1.40–2.92)	High	High	2.6 (1.50–4.53)
Throat irritation			High	Low	1.5 (0.77–3.01)
	Fungi	1.4 (0.93–2.09)	Low	High	1.6 (0.79–3.34)
	Endotoxin	1.5 (1.00–2.15)	High	High	2.2 (1.20–3.90)

[a]The models without interaction include demographic factors (age, gender, race, duration of occupancy, and smoking status), culturable fungi/m^2 floor, and endotoxin/m^2 floor.
[b]Interaction models include all the demographic factors, fungi and endotoxin/m^2 floor, and interaction between fungi and endotoxin. There are four interaction variables in these models – high exposure to both fungi and endotoxin; high exposure to fungi only; high exposure to endotoxin only; low exposure to both which is reference category.
[c]Floors in upper two tertiles were combined and categorized as high exposure floors for the dichotomous variables.
*Test *P*-value for fungi by endotoxin interaction effects = 0.18.
**Test *P*-values for fungi by endotoxin interaction effects <0.05.

associations between exposure to culturable fungi and endotoxin in floor dust in April 2002 and building-related respiratory, systemic, and skin symptom data collected in September 2001 are based on relative differences in exposure levels within the building. It is possible that historical levels of exposure were higher and resulted in sensitization to bioaerosol. After sensitization, occupants may react at lower levels of exposure. Remediation and carpet cleaning on all floors of the building may have lowered levels of culturable fungi and endotoxin proportionately, but may not have changed relative differences among the floors. On the contrary, before they were resurveyed in June 2002, new respiratory symptoms developed in about half of the participants who had been asymptomatic in the September 2001 survey (Cox-Ganser et al., 2005), suggesting that the lower levels of fungi and endotoxin documented in the April 2002 survey were associated with new-onset adverse health consequences.

Tertile fungal or endotoxin exposure variables based on cfu or EU per unit area of floor were better predictors for respiratory and other symptoms than cfu or EU per unit mass of dust, implying that cfu or EU per unit area better represent occupants' total exposure to bioaerosols or related contaminants than cfu or EU per unit mass of dust. This makes good sense because cfu or EU/m^2 area reflects both contaminant concentration (per unit mass of dust) and amount of dust load in the work stations.

Total fungi in chair dust had a weak positive association with total fungi in floor dust while endotoxin in floor and chair dust was not associated. There were similar findings in a study of microbial contamination of dust in a hospital setting (Rao et al., 2005), and it has been hypothesized that there may be different contamination sources for chairs and floors-for example, humans are a likely source of contamination for chair dust. In this study, although we do not fully understand the different contamination sources for the floor and chair dusts, we found floor dust to be more useful as an indicator of health risk than chair dust.

Interaction effect between fungi and endotoxin exposure

Our observation of interaction effects between endotoxin and fungi provides epidemiological evidence supporting the experimental findings of interaction effects. Endotoxin (a cell wall component of Gram-negative bacteria) and (1 → 3)-β-D-glucan (a cell wall component of fungi) are both potent pro-inflammatory

biological agents which produce non-allergic inflammation in airways (Douwes et al., 2002; Park et al., 2001; Rylander et al., 1998; Williams, 1997). Potential synergistic effects of (1 → 3)-β-D-glucan with endotoxin on inflammatory responses in airways (Fogelmark et al., 1994; Rylander and Fogelmark, 1994), on superoxide anion release from bronchial alveolar lavage (BAL) cells (Shahan et al., 1994), and on production of tumor necrosis factor-α (TNF-α; Ohno et al., 1995) were suggested from animal studies. Our data indicated that endotoxin exposure may modify the effects of fungal exposure (and vice versa) on respiratory symptoms (chest tightness, attacks of shortness of breath, and shortness of breath when hurrying) and that fungal exposure may require certain levels of co-exposure to endotoxin to produce a respiratory health effect and vice versa. In addition, our study findings imply that measuring both fungi and endotoxin may be critical in epidemiological studies examining the effects of fungal exposure on respiratory health.

Strengths and limitations

Two aspects of our exposure assessment methods for this study are noteworthy as strengths: First, we had a large number of dust samples taken throughout the building. The large sample size minimizes potential misclassification of exposure and thus decreases the possibility of underestimation of an effect toward the null (no association). Second, our methods of categorization based on rank order of individual floors based on the levels of biological agents in floor dust provided us with a robust tool for classifying exposure groups because potential measurement and sampling errors would be unlikely to substantially affect a rank-based categorization.

Although participation could not have been affected by quantitative environmental measurements conducted 7 months later, possible participation bias in responses to the questionnaire survey may represent a potential limitation of our study. Considering the history of water damage and health complaints in the building, employees who perceived their workstation to be water damaged may have been more likely to participate in the survey; similarly, asymptomatic employees who perceived no problem with their workstation may have been less likely to participate. To indirectly address this issue, we assessed whether participation rate differed by floor-exposure group for fungi and endotoxin using a generalized linear regression model. We did not find any significant association (P-values > 0.08) between participation rate and tertile exposure group for either fungi or endotoxin. An overestimation of the association between dustborne microbial agents and respiratory health might have occurred if the asymptomatic employees on floors with high levels of microbial agents (per m^2) in floor dust were less likely to participate in the study than asymptomatic employees on floors with low levels; it is not possible to assess the degree of this bias with our data because there were no symptom data collected from non-participants.

On the contrary, a healthy worker (survivor) effect may have also affected the study results as sicker employees may have no longer worked in the building and thus could not have participated in the study. However, such a healthy worker effect, if any, would have caused an underestimation of the association between dustborne microbial agents and respiratory health. Given our findings of significantly increased exposure-dependent responses for all lower respiratory symptoms, the fact that we found no association and no exposure–response relationships between fungal exposure and post-occupancy onset asthma with use of asthma medications in the past 12 months may be explained by this healthy worker effect.

Over- or under-reporting of symptoms by occupants who were aware of presence or absence of water damage in their work environments might also have affected our results. However, two arguments lessen concern about symptom reporting bias. First, categorization of occupants' exposure by the quantitative measurement of biological agents somewhat limits the effects of such reporting bias on the associations between objective exposure indices and health effects. Second, occupants' reported symptoms were validated in our previous publication, which found that spirometric and methacholine challenge test abnormalities and use of prescription medications substantiated symptom complaints in about two-thirds of those meeting an epidemiologic case definition of respiratory disease based solely on questionnaire responses (Cox-Ganser et al., 2005). Misclassification of individual exposure is also possible because we assigned the same exposure to all occupants on a floor by categorizing floors based on measurements of selected occupants' microenvironments. However, we were able to demonstrate positive exposure-related associations even with this potential misclassification. Communication among participants within each floor about their environment and possible health effects might have produced correlations in questionnaire responses, which might have reduced the size of standard errors of the estimates in our models.

In our study, environmental measurements were performed 29 weeks after the initial questionnaire survey. There was no repair or reconstruction of the building envelope between the time of the questionnaire survey and the time of the environmental sampling. Thus, the potential sources of dampness were unchanged. However, carpets were cleaned on all floors and replaced on one of the floors in this interim period. We conducted an environmental comparison

study before and 3 months after the replacement of carpet on that one floor by collecting floor dust at the same 55 sampling locations on both occasions. The total amount of dust collected was similar (GM before: 0.32 g; GM after: 0.37 g), and the level of total culturable fungi in floor dust after the carpet replacement was not substantially decreased (GM before: 14.6 cfu/mg; GM after: 10.4 cfu/mg; $P = 0.07$ testing for twofold or more reduction in the levels). Despite these environmental manipulations, which may have changed dust load or concentration of fungi and endotoxin in dust, our exposure categorization generally agreed with the previous history of water damage on floors, and we were able to demonstrate associations between culturable fungi and endotoxin in floor dust and health outcomes.

Selection of sampling locations was not random, but based on the workstations of severely symptomatic participants and of asymptomatic participants. This may have led to some bias in our estimates of floor-specific means of culturable fungi and endotoxin. However, the spatial distribution of sampling locations on a floor did not indicate any evidence of clustering of sampling locations in a certain area of the floors of the building, implying that our sampling locations were not much different from random sampling. If there were no true relationship between exposure level and health outcomes, then we could not have introduced systematic bias by our approach to selection of sampling locations; the estimated floor-specific means of fungi and endotoxin would not be systematically affected by the relative proportions of symptomatic to asymptomatic occupants. Therefore, it is unlikely that our findings reflect false positive relationships between exposure and health outcomes.

In conclusion, measured dustborne fungi and endotoxin levels in this water-damaged building were associated with respiratory and dermal symptoms in an exposure-dependent manner, consistent with a causal role of bioaerosols for those health effects in this building. A range of exposure within this building allowed us to examine exposure–response relationships in a setting with an excess of new-onset asthma following occupation of the building by the participants. Our exposure assessment method allowed us to address the deficits in epidemiologic evidence identified by the IOM report, allowing demonstration that mold and endotoxin are at least markers for exposures, if not causal agents themselves, for building-related respiratory symptoms characteristic of asthma, hypersensitivity pneumonitis, and rhinitis in damp indoor environments. Our finding that endotoxin exposure may change the effect of fungal exposure (and vice versa) on respiratory heath suggests that exposure to both fungi and endotoxin should be assessed in epidemiological investigations examining the effect of fungal or endotoxin exposure on respiratory health in indoor environments.

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